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Beneficial role of olive extract on cyclophosphamide-induced skin cellular injury

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ABSTRACT

For centuries, countries in the Mediterranean and all of Europe have employed olive leaves in traditional medicine. This substance has antioxidative stress and anti-inflammatory properties. In this work, the potential protective effects of olive extract were investigated in relation to the histochemical and histological alterations in the skin of rats following exposure to cyclophosphamide. Thirty-six mature male albino rats served as the subjects for the current study. There were three equal groups formed from them. The control group received no medical treatment. Animals in the cyclophosphamide group were given 100 mg/kg BW one dose of cyclophosphamide intraperitoneally. Rats in the third group received daily dosages of 15 mg/kg body weight of olive extract for one week prior to and one week following the injection of cyclophosphamide. Various stains were used to examine the histopathological as well as histo-chemical alterations in skin tissue. Cyclophosphamide-exposed rats' skin underwent a number of histological and histochemical alterations. Administering Olive Extract helped to improve these changes. The current study demonstrated olive extract's curative and preventive effects on albino rats' skin against cyclophosphamide-induced skin damage.

Keywords: Olive Extract, cyclophosphamide, Skin injury.

1. INTRODUCTION

The skin serves a variety of purposes, including regulating body temperature, excretion, absorption and sensory perception, as well as defense against injury, infection and dehydration. This tissue is composed of the dermis, an underlying connective tissue predominantly composed of dense fibrous components created by fibroblasts and the epidermis, an external stratified, non-vascularized epithelium. Due to their potent antioxidant activity, olive leaves have historically been utilized to treat a variety of medical ailments (Omar, 2010). Olive leaf has recently been reported to have anti-

inflammatory, antioxidant and diabetic properties. Oleuropein, the primary compound in olive leaves, is assumed to be in charge of the pharmacological actions. Oleuropein, a polyphenol that can lower high cholesterol and blood pressure, prevent cancer, shield cells from oxidative damage and slow cognitive decline and is the chemical that gives olive oil its variety of positive health effects (Hamdi and Castellon, 2005). Within 9 to 12 days of dosing, oleuropein totally regressed and suppressed many types of mouse tumors (Marchetti et al., 2015).

Cyclophosphamide is a harmful alkylating drug that is commonly used as an immunosuppressive medication for multiple sclerosis, systemic lupus erythematosus and other benign tumors as well as an antineoplastic agent for the treatment of different cancers (Kirkland et al., 1976). Treatment with cyclophosphamide causes biochemical and histological changes in the testis and epididymis of humans and rats, as well as oligospermia and azoospermia (Kaur et al., 1997; Masala et al., 1997). When used in both hazardous and therapeutic quantities, cyclophosphamide has been demonstrated to be deleterious to young rats' ability to heal wounds and maintain normal bone and skin growth. In one well-known study, it was discovered that cyclophosphamide did not impede mouse early wound healing regardless of dose or timing of administration (Wie et al., 1983). N-acetylcysteine other substance that can successfully prevent cyclophosphamide-induced alopecia when given parenterally or delivered topically in liposomes. In addition, imuVert plus N-acetylcysteine can be administered parenterally or topically to prevent the alopecia that cyclophosphamide and cytarabine can produce (Jiménez et al., 1992).

This study, to our knowledge, is one of the few that assesses the preventive qualities of olive extract against the skin damage that cyclophosphamide causes in rats. This research of olive extract protective properties on cyclophosphamide-induced skin damage in rats was the goal of the study.

2. METHODS

The PSA University Ethical Committee accepted the Animal Research Guideline for the Use and Care of Animals in Research, which we adhered to in our study, Al-Kharj (PSAU-2022 ANT 88/43PI). The study, which lasted for nine months from May 2022 to January 2023, was experimental. Weighed and finely ground olive leaves were used. Two times (each for 24 hours) the powdered plant material was extracted in 70% ethanol using soxhlet equipment. For two weeks, the treatment group received one intragastric gavage (fifteen mg/kg B.W.) dose per day of olive leaf extract (Mohammadi and Naik, 2008).

The Animal Unit of PSA University provided a total of thirty-six (130 ± 5 gm) male Swiss albino rats. They were monitored for 15 days while they acclimated in the lab. They were kept in plastic cages in a group setting under normal lighting, ventilation, temperature and humidity conditions. Food and water were freely available to animals. From Frankfurt, Germany's Baxter Oncology GmbH, cyclophosphamide was purchased.

Three groups of the test animals were created at random; 12 animals in each group. Normal, healthy rats in the control group received no therapy. Rats in the cyclophosphamide group were given intraperitoneal administration of one dose of cyclophosphamide (100 mg/kg 1 B/W). Our investigation used a 100 mg dose of cyclophosphamide, but prior to the trial, we gave 100 mg kg 1 BW of cyclophosphamide to five rats to ensure that we could see the major histological changes in the skin. One week prior to and one week following cyclophosphamide administration, the last group of rats in this group received daily treatment with olive leaf extract fifteen mg/kg body weight.

The animals were fasted for the whole duration of the administration, given water as needed and then sedated with pentobarbital sodium (35 mg/kg, i.p.) before being terminated by cervical dislocation seven days following cyclophosphamide administration. Extracted skin samples were fixed in neutral formalin solution 10%. Following standard processing, 4-m-thick paraffin slices were created for the histological and histochemical analyses using hematoxylin and eosin. Mallory's trichrome stain was used to color collagen fibers. On the other hand, the mercury bromophenol blue technique was used to identify total proteins (Suvarna et al., 2018). The mean and standard deviations were used to express all values. Using the statistical program SPSS 13.0, a student's t-test was used for the statistical analysis.

3. RESULTS

The skin sections from the control group exhibited normal histological characteristics of the dermis and epidermis, including thin epithelium, evenly spaced glands and undamaged hair follicles in the dermis. Four layers of keratinocytes made up the epidermis. There were many capillaries and connective tissue cells in the dermis' papillary layer. In contrast, a dense fibrous connective tissue made up the inner reticular layer. The arrector pili muscle encircled the sebaceous, sweat and hair follicles in the dermis (Figure 2A, B, C, D).

The skin samples from the cyclophosphamide group displayed a wide range of pathological conditions. Epidermal cells stopped growing, hair follicles and sebaceous glands disappeared, dermal cells swelled and collagen fibers became edematous. Epidermal cells had nuclear pyknosis and karyolysis, corneum separation and an unorganized papillary layer. Skin samples from the olive extract group showed some epidermal and dermal structure that had been restored to its normal tissue pattern (Figure 2A, B).

Mallory's trichrome stained sample from the control group displayed collagen fibers with a typical appearance and organization that were blue-stained. Just below the epidermis' basal lamina, small clumps of collagen fibers were seen. Collagen fibers emerged as a loosely distributed network in the papillary layer of epidermis. Collagen fibers increased in number and joined formed thick, asymmetrical bundles in the reticular layer (Figure 2C). In the basal lamina, in reticular layers and the dermal papillary of the cyclophosphamide group, irregular, densely organized collagen fibers have been identified (Figure 3C). Collagen fiber concentration in the olive extract group significantly decreased in contrast to the cyclophosphamide one (Figure 4C).

Investigation of normal skin samples stained with mercury bromophenol blue revealed total protein normal distribution of within the control group. Deeply to moderately stain cells of the dermal, papillary and basal laminae, as well as reticular layers represented this typical distribution (Figure 1). The epidermis basal lamina within the cyclophosphamide group showed a significant increase in the amount of total protein, but the dermal papillary and reticular layers showed a decrease (Figure 3D). The epidermis basal lamina in the olive extract group showed elevated levels of total protein; whereas the dermal papillary and reticular layers showed lower levels (Figure 4D).

In the skin slices from the control group that had been DNA granules of a magenta hue indicated a normal allocation of DNA content in the nuclei of dermal and epidermal layer cells. DNA content was found to have significantly increased in the epidermal and dermal layer nuclei of cyclophosphamide group. In the Olive Extract treated group, DNA content in epidermal and dermal cell nuclei appeared to be more or less normal (Table 1) (Figure 1). The epidermis basal lamina, as well as the dermal reticular and papillary layers, displayed a modest PAS reaction in the cyclophosphamide group. Compared to the cyclophosphamide group, the staining harmony of the epidermal basal lamina and the dermal reticular and papillary layers was mild in the Olive Extract group of animals (Table 1) (Figure 1).

Table 1 Demonstrating DNA content in the skin of the control and treatment groups and MOT values of PAS +ve materials

		Control Group	Cyclophosphamide Group	Olive extract Group
DNA content	Mean	71.3	37.8	59.88
	SD	15.38	29.77	20.11
	%	4	3	-13.90
	t Test		0.001	0.002
PAS +ve	Mean	89.99	87.43	89.14
	SD	19.23	34.87	15.77
	%		-19.33	-633
	Mean	97.34	98.9	95.04

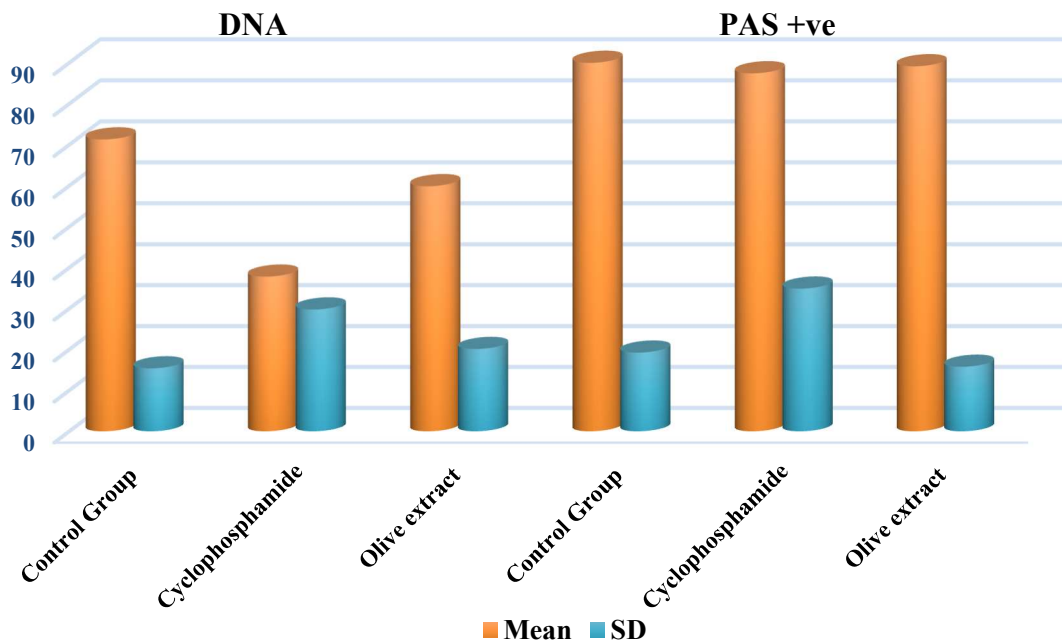


Figure 1 Displaying DNA and MOT values of PAS +ve content in the skin of different groups

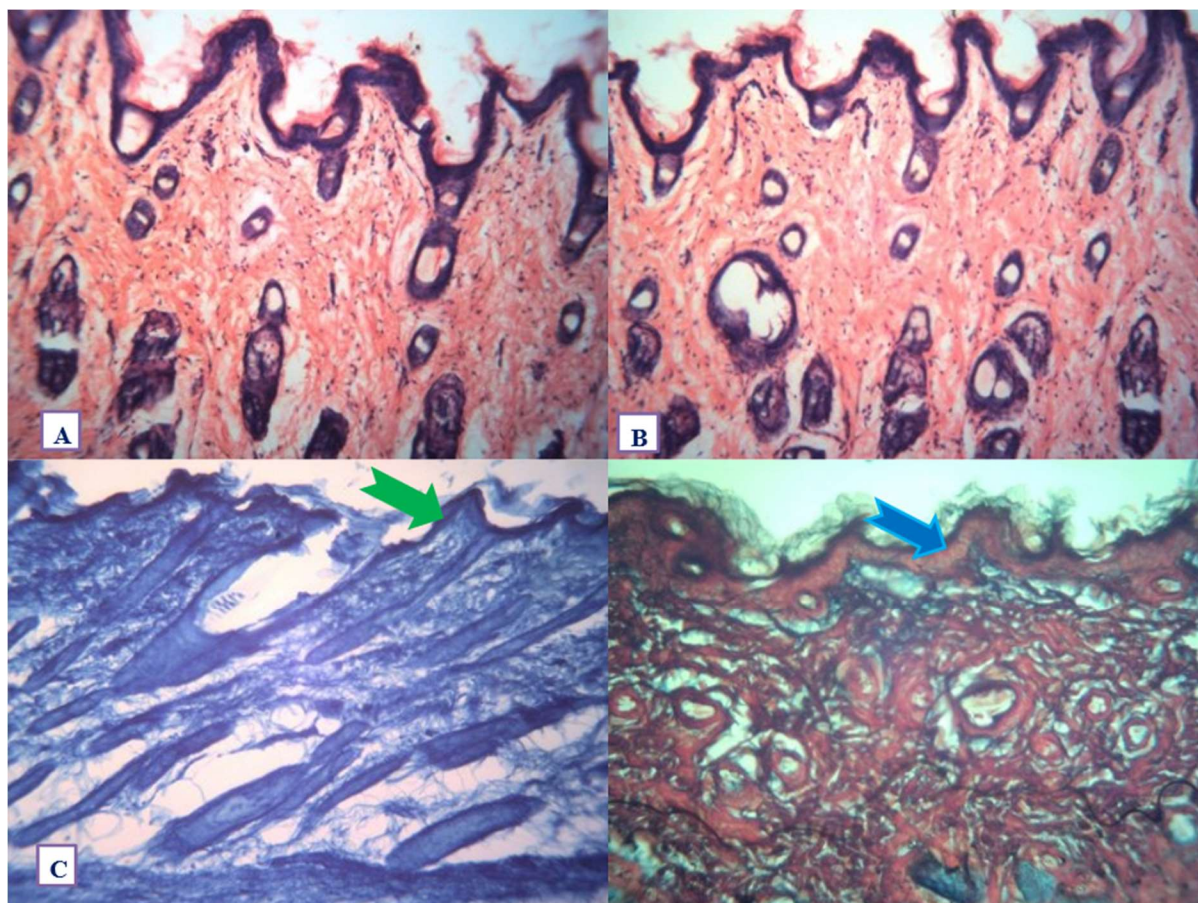


Figure 2 A, B) HE stain of the control group's histopathology findings was normal. C) Mercuric bromophenol blue shows normal distribution of total protein in the control albino rat's skin (green arrow). D) Mallory's trichrome exhibiting collections of tiny collagen fibers beneath the epidermis' basal lamina (blue arrow)

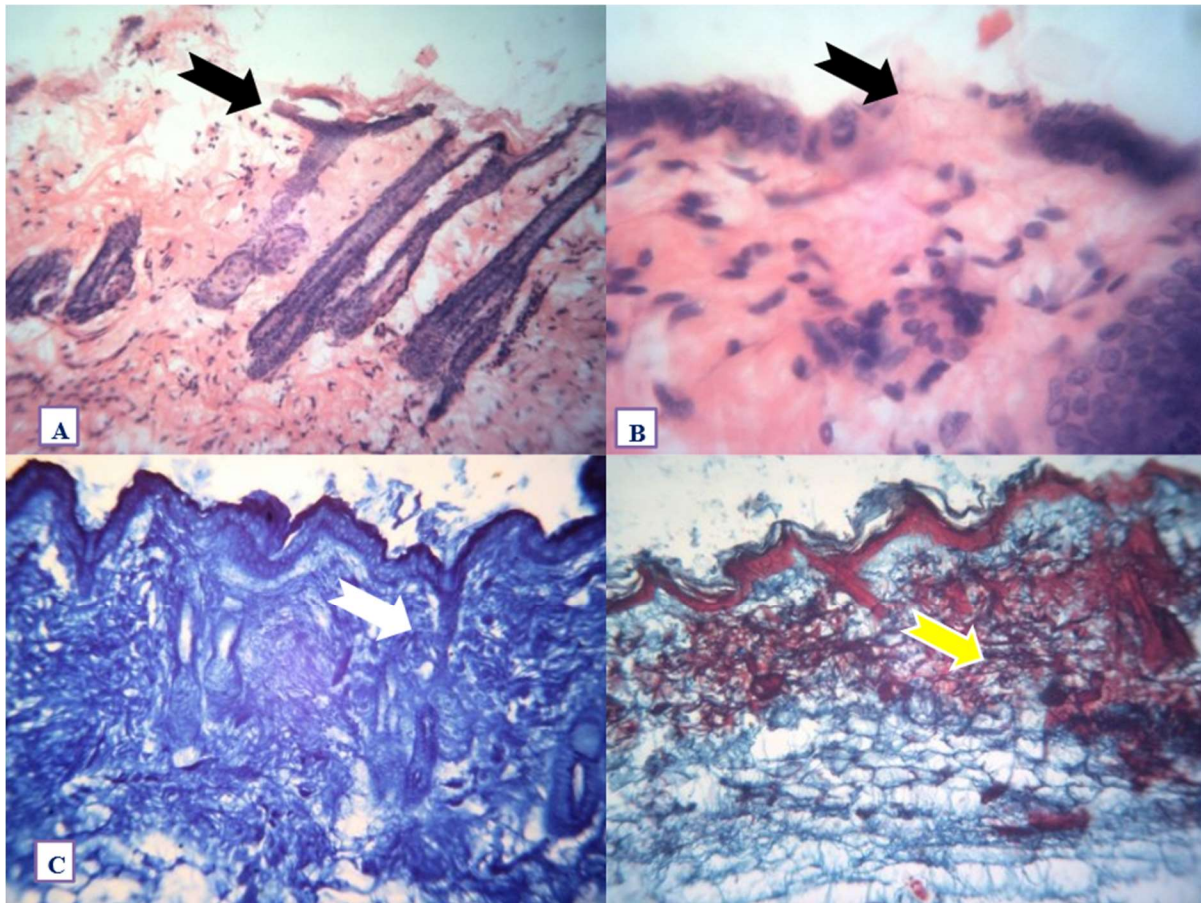


Figure 3 A, B) The HE results from the cyclophosphamide group demonstrate deteriorated hair follicle epithelial cells organization problem as discontinuous epidermal and dermal cells (black arrow). C) Using mercuric bromophenol blue, the amount of total protein is seen to have significantly increased (white arrow). D) Mallory's trichrome showing many, irregularly organized, thick collagen fibers (yellow arrow)

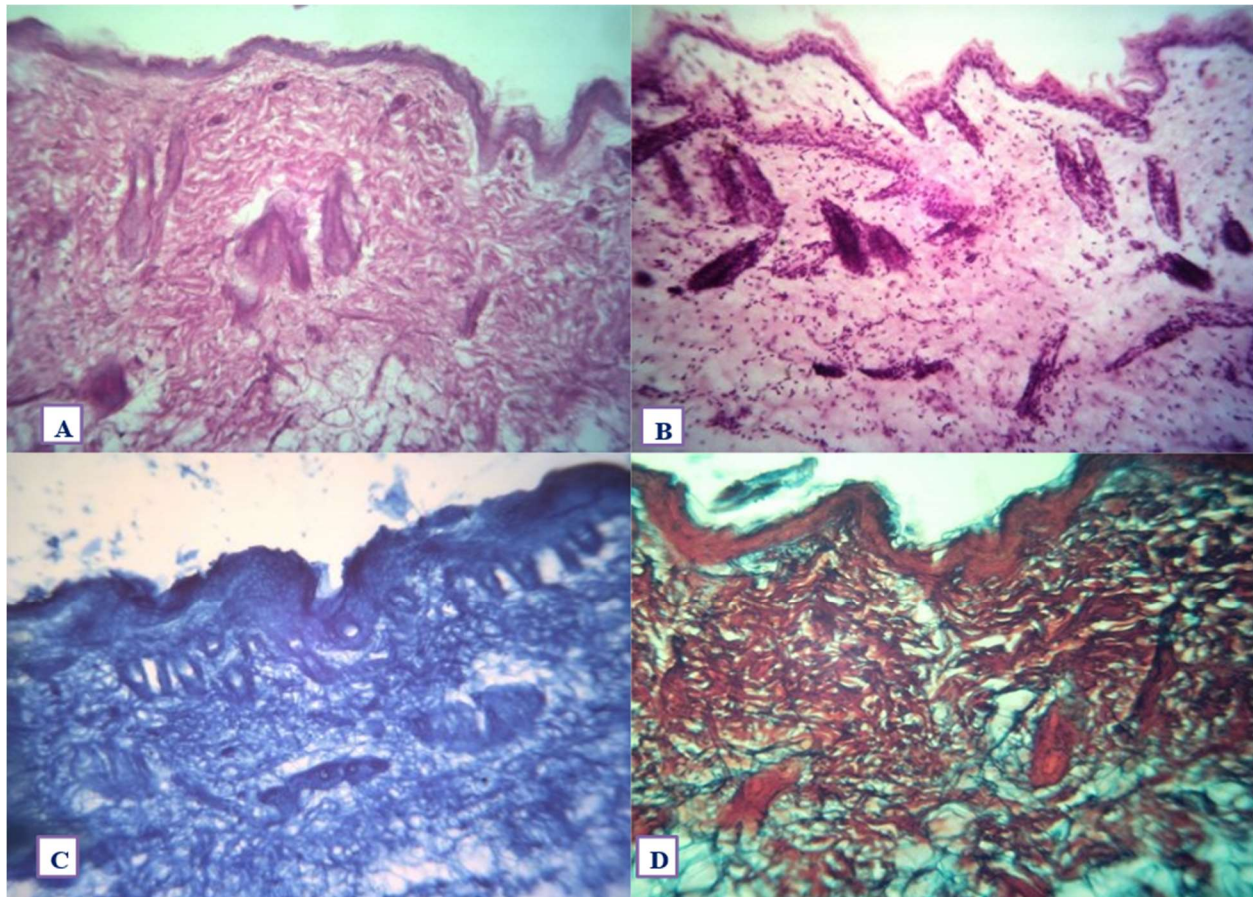


Figure 4 A, B) The H.E results of the olive extract group's histopathology findings was normal. C) Increasing the amount of total protein in the epidermis' basal lamina using mercuric bromophenol blue. D) Mallory's trichrome reveals a noticeable drop in the amount of collagen fibers

4. DISCUSSION

This investigation into the shielding properties of olive extract on rat skin after cyclophosphamide injection was the goal of the study. According to our knowledge, this study is one of the few that investigates the antioxidant properties of this extract in relation to the skin damage that cyclophosphamide causes in rats.

Our findings concur with those of a related study that found olive oil, green tea, ginseng and chamomile, to be helpful in the treatment of skin lesions (Shabanian et al., 2017; Pazyar et al., 2014). These medicinal herbs have been employed mostly for the healing of cutaneous wounds because they have high phytotherapeutic effects and significant pharmacological effects. Olive oil treatment reversed the loss in collagen deposition and fibroblast migration, reduced lipid peroxidation and enhanced cutaneous wound healing in chronically stressed mice (Rosa-Ados et al., 2014). This improvement in the current study may be attributable to the action of oleuropein, the most prevalent phenolic compound in olive leaves, which has been shown to have healing effects on damaged skin by enhancing collagen production, accelerating epithelial formation and increasing blood flow to the injured area (Mehraein et al., 2014).

Following the application of olive extract, improvements were seen in the PAS +ve materials, total protein, DNA and mast cell contents as compared to the cyclophosphamide group. This enhancement might be the result of olive leaf extract's effects on the tissue's DNA mending machinery and protein synthesis. Oleuropein also functions as a free radical scavenger since DNA components are their primary target. The antioxidant properties of olive leaf extract, where oleuropein promotes the synthesis of mRNA and protein as well as endothelium, may also contribute to this improvement (Carluccio et al., 2003). Numerous in vivo and in vitro studies that demonstrate the variety of advantageous properties of oleuropein and its derivatives have piqued the interest of researchers in these substances. This has helped to identify and characterize additional biological activities of oleuropein with functional, pharmacological and biomedical potential. Natural ingredients and plants have established themselves as a dependable and significant source for the creation of novel medications. It has been demonstrated that olive extract significantly reduces the risk of liver cirrhosis and hepatotoxicity brought on by methotrexate (Al-Attar and Shawush, 2014). Olive oil can dramatically decrease

fluoxetine-induced hepatotoxicity and lower inflammation, oxidative stress and apoptosis in rats, according to a recent study (Elgebaly et al., 2018).

5. CONCLUSION

According to the findings of the current investigation, olive treatment has a positive therapeutic effect against the histochemical and histological changes caused by cyclophosphamide in the skin of rats. These have a preventing effect on skin tissue damage, which may help lower the likelihood of developing new ailments.

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Authors' Contributions

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A Ali, Salman Bin Dayel and Mansour M Alajmi participated in the study design and wrote the first draft of the manuscript. Turki Saad Alsubaie, Emad M Al-Otaibi, Nasser Hassan Al-swedan, Maan Omar Alzuhairi and Musab Alrezehi collected and processed the samples. Roaa Salem Alharbi, Abdullah N Al-Khanfoor and Nawaf Saad Alarfaj participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

Ethics Approval

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (PSAU-2022 ANT 88/43PI).

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Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

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